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COMPLETE SPECIFICATION

Water-soluble Anionic Polysaccharide Derivatives

We G. D. SEARLE & Co. a Corporation organised and existing under the laws of the State of Delaware, United States of America, of Post Office Box 5110, Chicago 80, Illinois, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to water-soluble anionic polysaccharide derivatives and a process for their preparation.

Starch as it is obtained from any of the usual sources such as potatoes, contains two distinct and separable components. They are amylose, characterised by linear chains only, and amylopectin, possessing both linear and branched-chains. The physiochemical characteristics of each component are dependent upon the source and also upon the fractionation method used for its separation. A specific characteristic contemplated is the size of the molecule, indicated, for example, by measurement of molecular weight and molecular shape. Molecular weight data are obtained by light scattering-measurements. In addition, further characterisation is obtained by viscosity measurements.

In the amylopectin molecule, the linear chains containing repeating glucose units joined by 1,4-glycosidic linkages, participate in branch formation, wherein they are typically cross-linked by 1,6-glycosidic linkages. Those glucose units having only the 1,4-linkage possess three potentially reactive hydroxyl groups, while those units possessing also the 1,6-linkage have but two hydroxyl groups available for reaction. In view of the fact that only about 2—4% of the glucose units in amylopectin are connected by branched-chain linkages, however, it is apparent that there is an average of almost three potentially reactive hydroxyl groups per glucose unit. Thus, varying degrees of substitution can be achieved by control of the extent to which available hydroxyl groups are esterified. By the process of the present invention sulphated amylopectins having varying degrees of substitution (i.e. substantially 1 to 1.8 sulphate groups per glucose unit) have been produced.

The present invention provides pepsin-inhibiting an anti-ulcerogenic sulphated amylopectins, containing from one to 1.8 sulphate groups per glucose unit, and the water-soluble salts thereof, said sulphated amylopectin products having a molecular weight of the order of 1 to 30×10^5 and being derived from potato starch amylopectin, characterised by a molecular weight greater than 1×10^5, which has suffered minimum degradation by sulphation under conditions causing minimal degradation. The sulphated amylopectins and their salts provided by the present invention have the property of high and rapid solubility in water.

The present invention also includes a process for the production of the pepsin-inhibiting and anti-ulcerogenic sulphated amylopectins and the water-soluble salts thereof of the present invention wherein potato starch amylopectin, which has suffered minimum degradation and is characterised by a molecular weight greater than 1×10^5 is contacted with a sulphating agent known to sulphate hydroxyl groups under conditions causing minimal degradation until 1 to 1.8 sulphate groups per glucose unit has been introduced.

Preferably the sulphation is allowed to proceed to a degree of substitution of substantially 1.6 sulphate groups per glucose unit.

In choosing the amylopectin starting material it has been determined that molecular weight and degree of depolymerisation suffered during the fractionation process are particularly significant criteria. A molecular weight in the range of $1—30 \times 10^5$ is preferred. (The expression 'molecular weight' as used herein refers to weight-average molecular weight.). For the purpose of the present

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invention, amylopectin derived from potato starch (for example, by selective precipitation with certain inorganic salts as described in Specification No. 722,586) is a preferred starting material, apparently due to its molecular weight which is of the order of 6×10^7 and being in the optimum range. The preferred sulphated products of this invention are characterised by a molecular weight in that same range as a result of sulphating conditions involving minimum degradation. Starch fractions of lower molecular weight (e.g. amyloses or amylopectins derived from maise as well as those containing greater proportions of amylose) produced products which are less desirable for the purposes of this invention.

In the practice of the present invention, it has been found that the extent of sulphation of the amylopectin molecule can be controlled by selection of the appropriate reaction conditions, including such factors as: choice of sulphating agent ratio of sulphating agent to polysaccharide, time, temperature and pH of the reaction mixture. Of the variety of agents which can be used for the sulphation are: chlorosulphonic acid, alkali metal chlorosulphonates, sulphamic acid, sulphuryl chloride, gaseous sulphur, trioxide, a mixture of sodium nitrite and sodium bisulphite, and complexes of sulphur trioxide with an organic base [e.g. sulphur trioxide-trimethylamine, sulphur trioxide-triethylamine, sulphur trioxide-pyridine, sulphur trioxide-dimethylaniline and sulphur trioxide-dioxane, and sulphur trioxide-bis(2-chloroethyl)ether]. With the selection of the appropriate sulphating agent, corresponding reaction conditions (such as time, temperature, pH and solvent) known to one skilled in the art will suggest themselves. For example, one skilled in the art would recognise the necessity of reducing the extreme hydrolytic or degrading effect of certain sulphating agents by utilising a corrective solvent, e.g. chlorosulphonic acid can suitably be used in pyridine or formamide. Use of a large excess of a vigorous sulphating agent, for example, chlorosulphonic acid under the conditions described by P. Bernfeld *et al.*, *J. Biol. Chem.*, 235, 2852 (1960) should be avoided since this affords compounds which are not water-soluble and thus undesirable for the purposes of this invention.

A particularly useful sulphating agent is the sulphur trioxide-trimethylamine complex. The procedure utilising this reagent can be conveniently carried out in water, in the presence of alkali, and preferably at a temperature from 0° C. to 100° C., inclusive, for a period of 2 to 100 hours. Since temperature and time are interpendent, use of a lower reaction temperature will require a longer reaction time. The compounds produced by the conditions described herein do not present spectophotometric evidence of sulphur cross-linkage.

Equivalent to the amylopectin sulphates of the present invention are the pharmaceutically acceptable non-toxic salts thereof, conveniently formed with a variety of metallic cations, ammonium ions, and organic bases. Such salts are formed with metallic cations such as: alkali metal cations—preferably, sodium, ammonium ions; and with organic bases such as: amines—suitably, trimethylamine and triethylamine, and with related salts.

Suitable procedures for the isolation and purification of the sulphated amylopectins and salts thereof of this invention include the following utilised separately or in combination: centrifugation; lyophilisation, i.e. freeze-drying; dialysis; drying; alcohol precipitation; and precipitation with quaternary ammonium salts.

The sulphated amylopectins and salts thereof (containing substantially one to 1.8 sulphate groups per glucose unit) of the present invention are useful by reason of their valuable pharmacological properties. For example, they possess a high degree of potency as pepsin inhibitors and anti-ulcerogenic agents. Particularly potent are the alkali metal and ammonium salts of the present invention, which are also preferred for their enhanced stability and rapid water-solubility. It has been found that, in general, conditions leading to those salts having more than 1.8 sulphate groups per glucose unit produce products that are significantly less water-soluble and thus less desirable.

Of the salts of the present invention the sodium salts (containing 12—16.5% of sulphur) are especially good pepsin inhibiting and anti-ulcerogenic agents.

A particularly preferred and useful embodiment of this invention is the sodium salt of sulphated potato starch amylopectin possessing substantially 1.6 sulphate groups per glucose unit and characterised by a molecular weight of about 6.3×10^7.

The compounds of the present invention have also been found to exhibit moderate anti-coagulant activity and negligible anti-lipemic activity.

In the following examples, temperatures are given in degrees centigrade (° C) and quantities of materials are expressed in parts by weight unless otherwise noted.

EXAMPLE 1

To a suspension of 100 parts of potato starch amylopectin in 200—800 parts of water was added successively 25—50 parts of 10%—50% sodium hydroxide and 100—400 parts of the sulphur trioxide-trimethylamine complex. The reaction mixture was stirred vigorously for about 12—24 hours at room temperature, and then at 40—60° for 1—15 hours.

The resulting mixture was then purified by one, or a combination of the methods hereinbefore described to afford the sodium

salt of sulphated potato starch amylopectin containing substantially one to one and three-fourths sulphate groups per glucose unit.

Use of 100 parts of potato starch amylopectin, 600 parts of water, about 35 parts of 10% sodium hydroxide and 300 parts of the sulphur trioxide-trimethylamine complex in the above procedure for a period of 18 hours at room temperature, followed by 5 hours at 50°, yielded after dialysis and spray drying, the sodium salt of sulphated potato starch amylopectin containing substantially 1.6 sulphate groups per glucose unit, as a powder. This substance is characterised by a molecular weight of approximately 6.3×10^7 as determined by the light-scattering method. A 0.5% solution in distilled water, at 20° C., exhibits an absolute viscosity of 11.25 centipoises when measured by the method of Höppler, *World Petroleum Congress London Proc.*, 2, 503 (1933).

In a similar manner, substitution of a molecular equivalent quantity of ammonium hydroxide for sodium hydroxide used in the above procedures afforded respectively: the ammonium salt of sulphated potato starch amylopectin possessing substantially one to one and three-fourths sulphate groups per glucose unit, and the ammonium salt of sulphated potato starch amylopectin containing substantially 1.6 sulphate groups per glucose unit.

EXAMPLE 2

To about 75 parts of pyridine was added, at 10—20° over a period of about 2 hours, 12 parts of chlorosulphonic acid. After completion of the addition, the mixture was heated to about 70°, and 2 parts of potato starch amylopectin were added with stirring. Heating and stirring were continued for about 2 hours, after which time agitation was stopped, and the mixture was allowed to stand in order to permit the product to settle. The hot supernatant layer was removed by decantation and discarded. To the solid was added approximately 70 parts of water, and then concentrated hydrochloric acid to approximately pH 2. The resulting aqueous acidic mixture was added, with vigorous stirring, to about 165 parts of 2-propanol. At the end of approximately 30 minutes, stirring was discontinued, and the precipitate was allowed to settle. The supernatant liquid was decanted and discarded. Approximately 60 parts of water were added to the precipitate, and this aqueous mixture was stirred until solution was complete. To that solution was carefully added 10% aqueous sodium hydroxide to about pH 6.25, and this alkaline solution was added to about 165 parts of 2-propanol. After stirring for about 10 minutes, the precipitate was allowed to settle, and the 2-propanol phase was separated by decantation and discarded. To the precipitate was then added approximately 40 parts of water, and the resulting mixture was stirred for about 16 hours. The pH was adjusted to approximately 8.5 by the addition of 10% aqueous sodium hydroxide, and this alkaline solution was added to about 165 parts of 2-propanol at a temperature of about 10°. After stirring for about 15 minutes and standing for about 10 minutes, the supernatant 2-propanolic solution was decanted, and the precipitate was stirred with about 60 parts of water. This aqueous mixture was clarified by centrifugation, then was spray dried to afford the sodium salt of sulphated potato starch amylopectin as a white powder. The product contains substantially 1.8 sulphate groups per glucose unit and possessed a molecular weight of about 125 million, as determined by light-scattering technique.

EXAMPLE 3.

To 284 parts of pyridine was added dropwise with stirring 10 parts of chlorosulphonic acid, keeping the temperature at about 50°. The temperature was then raised to about 77°, and 7 parts of potato starch amylopectin were added. Stirring and heating were continued for approximately 1 hour, at the end of which time the reaction mixture was allowed to stand in order to permit the insoluble material to settle. The supernatant pyridine solution was removed by decantation, and the remaining solid was dissolved in 200 parts of water. This aqueous solution was made acidic to pH 1.6 by the addition of concentrated hydrochloric acid, and then was poured into 1280 parts of ethanol. The resulting mixture was allowed to stand at 0—5° for about 16 hours. Decantation of the supernatant liquid afforded a solid residue which was dissolved in 200 parts of water. This aqueous solution was made alkaline to a pH of approximately 9 by the addition of dilute sodium hydroxide. Lyophilisation of a portion of this pH 9 aqueous solution afforded the sodium salt of sulphated potato starch amylopectin containing substantially 1.2 sulphate groups per glucose unit.

The remainder of that aqueous alkaline solution was centrifuged at 3600 revolutions per minute for about 90 minutes, and the supernatant liquid dried by lyophilisation, resulting in the sodium salt of sulphated potato starch amylopectin containing substantially 1.6 sulphate groups per glucose unit.

Lyophilisation of a suspension of the residue afforded the sodium salt of sulphated potato starch amylopectin containing substantially one sulphate group per glucose unit.

EXAMPLE 4

To a suspension of 100 parts of potato starch amylopectin in 400 parts of water was added successively 35 parts of 15% sodium hydroxide in 300 parts of the sulphur trioxide-trimethylamine complex. The reaction mixture was stirred vigorously for about 26 hours

at room temperature and then at 48° for 14 hours. To the neutral solution was added cetyltrimethylammonium chloride. The resulting precipitate was removed by centrifugation and the precipitating quaternary ammonium derivatives was removed by suspension of the precipitate in water, and then dried to afford the cetyltrimethylammonium salt of sulphated potato starch amylopectin containing substantially 1.5 sulphate groups per glucose.

Other quaternary ammonium compounds can be substituted for ceyltrimethylammonium chloride in the process of Example 4 with similar results.

WHAT WE CLAIM IS:—

1. Pepsin-inhibiting and anti-ulcerogenic, sulphated amylopectins, containing from 1 to 1.8 sulphate groups per glucose unit, and water-soluble salts thereof, said sulphated amylopectin products having a molecular weight of 1 to 30×10^7, and being derived from potato starch amylopectin characterised by a molecular weight greater than 1×10^7 and which has suffered minimum degradation, by sulphation under conditions causing minimal degradation.

2. Pepsin-inhibiting and anti-ulcerogenic sulphated amylopectins, and the water-soluble salts thereof, as claimed in claim 1, derived from potato starch amylopectin, which has suffered minimum degradation, by sulphation with the sulphur trioxide-trimethylamine complex under conditions causing minimal degradation.

3. Pepsin-inhibiting and anti-ulcerogenic water-soluble salts of sulphated amylopectins as claimed in claim 1 or 2, wherein the salts are the alkali metal and ammonium salts.

4. Pepsin-inhibiting and anti-ulcerogenic water-soluble salts of highly and rapidly water-soluble sulphated amylopectins, as claimed in claim 3 wherein the alkali metal is sodium.

5. A pepsin-inhibiting and anti ulcerogenic water-soluble sodium salt of sulphated potato starch amylopectin, as claimed in any one of the preceding claims, said sodium salt containing substantially 1.6 sulphate groups per glucose unit and characterised also by a molecular weight of about 6.3×10^7.

6. A pepsin-inhibiting and anti-ulcerogenic water-soluble sodium salt of sulphated potato starch amylopectin, as claimed in any one of preceding claims 1 to 4, said sodium salt containing substantially 1.8 sulphate groups per glucose unit and characterised also by a molecular weight of about 12.5×10^7.

7. A process for the production of pepsin inhibiting and anti-ulcerogenic sulphated amylopectins and the water-soluble salts, thereof as claimed in any one of the preceding claims, wherein potato starch amylopectin, which has suffered minimum degradation and is characterised by a molecular weight greater than 1×10^7, is contacted with a sulphating agent known to sulphate hydroxyl groups under conditions causing minimal degradation until 1 to 1.8 sulphate groups per glucose unit have been introduced.

8. A process as claimed in claim 7 wherein the sulphating agent is sulphur trioxide-trimethylamine.

9. A process as claimed in claim 7 or 8 wherein the salts are the alkali metal and ammonium salts.

10. A process as claimed in claim 9 wherein the alkali metal salt is the sodium salt.

11. A process as claimed in any one of the preceding claims 7 to 10 wherein the sulphation is allowed to proceed to a degree of substitution of substantially 1.6 sulphate groups per glucose unit.

12. A process as is claimed in any one of the preceding claims 7 to 10 wherein the sulphation is allowed to proceed to a degree of substitution of substantially 1.8 sulphate groups per glucose unit.

13. A process as claimed in claim 7 substantially as described with reference to any one of the Examples.

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